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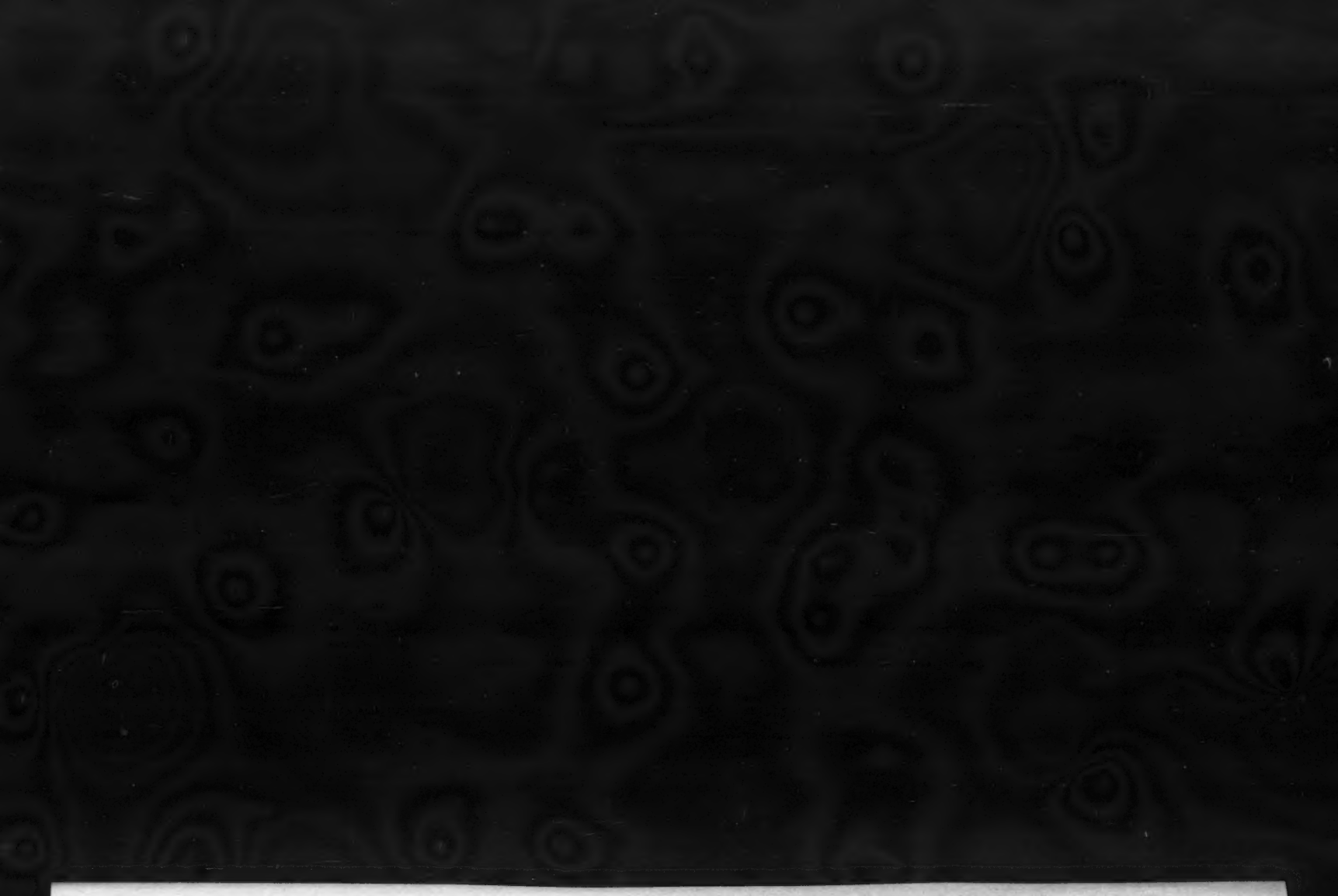

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THE EFFECT OF INCREASED VITAMINS IN THE EGG ON THE DEVELOPMENT OF THE CHICK

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(Received for publication on March 20, 1947)

INTRODUCTION

Although extensive investigations have been conducted on the nutritional requirements of growing and mature animals, relatively little is known of the vitamin requirements of embryos. This is probably due to the difficulty in controlling the food supply of many embryos, which makes them undesirable for such a study. Since its food supply is limited to the yolk and albumen of the egg up to the twenty-first day, time of hatching, the chick embryo can be used in this work to greater advantage. Moreover, the vitamin content of the egg can be altered by the controlled feeding of the hen laying the egg and by additions of vitamin and pro-vitamin substances to the yolk and albumen through injection.

Much of the present information on deficiency diseases has been obtained from experiments on the growing chick. It has been demonstrated in a few of these investigations that pro-vitamin substances have vitamin activity. Thus, Capper, McKibbin, and Prentice (1939) were able to raise chickens to maturity on a synthetic diet to which either carotene or cod-liver oil concentrate was added. Chicks fed the basal ration plus carotene showed satisfactory growth and contained vitamin A in their livers. Kline, Schultze, and Hart (1932) have also demonstrated that carotene is a source of vitamin A for the growing chick, and Frohring and Wyeno (1934) have shown that the addition of carotene to a ration free of vitamin A delays the appearance of deficiency symptoms and prolongs life in direct proportion to the amount of carotene or of vitamin A added to the diet. Ahmad (1933) showed that the hen is second to the rat in capacity to synthesize vitamin A from carotene. This capacity to convert carotene into vitamin A has been demonstrated in other organisms.

The study of the effects of vitamins on ontogeny has also been pursued in other groups. In mammals the study is made difficult by the fact that the nutrition of the embryo depends upon activities within the mother and information as to maternal contribution in terms of specific materials quantitatively estimated is difficult to obtain. In the chick some of these difficulties are not present. For that reason, this study was undertaken in an effort to relate known vitamin deficiencies and excesses to specific morphological modifications in the development of the chick embryo. Eggs with a deficiency of certain vitamins can be obtained by controlling the diet of the hen producing the egg, and excesses of vitamins can be effected by the direct addition of substances of known vitamin content by injection.

Several investigators have employed the injection method in studying the influence of various substances upon the chick embryo. Surrendre (1934) studied the effect upon growth of the embryo following the injection of vitamin C into the egg. This method was used in studying the effect of hormones upon the embryo by Breneman (1935), by Mme. Guelin Schedrina (1934), by Malespine (1929), and by Willier, Gallagher, and Kock (1937). Dyes of various types have been injected into the developing egg of the hen by Burnier and Sauser-Hall (1935), by Hanan (1937), by Heine (1936) and by Latta and Busby (1929). Franke, Moxin, and Tully (1936) produced monstrosities by the injection of selinium salts into the developing egg, while Gallera (1934) produced abnormalities by introducing aid bubbles under the area pellucida. Morita (1936) has induced monsters, twins and multiple embryos by injections of dead organ substances from the embryo of the frog, the chick, and the mouse (organizer materials).

Catizone and Gray have demonstrated that lead injected into the subgerminal cavity of the chick embryo produces abnormalities of the central nervous system. Taylor, Thacker, and Pennington (1941) increased the amount of pantothenic acid available to the chick embryo by injection before incubation of the egg and observed that low concentrations are associated with a larger than normal brain, while higher concentrations depressed the development of the heart and brain.

In the experiments to be described below, substances of known vitamin content or their precursors were injected into the subgerminal

cavity of the embryo and the effects upon the developmental pattern were recorded for each of the specific substances used.

I am indebted to Professor Leigh Hoadley for advice and criticism; thanks are due also to Professor A. B. Dawson and to Dr. George Wald for suggestions during the course of the work.

Materials and Method

Eggs from pure-bred Rhode Island hens were obtained from inbred selected flocks near Cambridge, Massachusetts and from the Bureau of Animal Industry, Department of Agriculture, Washington, D. C. In all, about 700 eggs were used. The eggs were incubated at a temperature of 38-40° C., and an allowance of four hours was made in computing temporal ages. To make an injection, the egg was placed in a syracuse watch-glass containing a wad of cotton and the shell was washed in Lugol's solution. A window about 7 mm. square was cut in the shell with a hack saw blade. The shell-membrane was opened, exposing the blastoderm and the injection was made through the vitelline membrane into the perilecithal space with a no. 27 diphtheria syringe needle. The needle was sealed to the end of a capillary pipette with Dekotinski cement. To the other end of the pipette was attached a piece of rubber tubing and a rubber bulb by means of which the necessary pressure for injection was applied. All instruments were sterilized either by washing with 95% alcohol or by heating over a flame. Injections were made after an incubation period of 24 hours. With care during manipulations, harmful effects resulting from mechanical disturbances can be avoided (cf. Gallera, 1934). Injections of this type seldom impede development or produce abnormalities (cf. Malespine, 1929).

Table 1 shows the vitamins and pro-vitamins used and the concentration of each in terms of U.S.P. units.

Un-injected embryos and embryos treated with cotton-seed oil and nujol were used as controls. Following injections, the shell-membrane and shell were replaced and the edges of the shell were sealed with warm paraffin (56°). The blastoderm thus comes to lie below an area of the intact shell. Twenty-four and forty-eight hours after injection the oils may be located immediately below the surface of the blastoderm. Preliminary examinations were made in warm Ringer's fluid (38.5°) while the embryo was still on the yolk. Blastoderms were fixed in Helly's solution, and stained in borax carmine.

TABLE 1

	Initial Concentration (U.S.P. units/gram) Vit. A	Vit. D	No. Drops 1 dr.—5/1000 cc	U.S.P. Units Vit. A	Vit. D
Cod-liver Oil	1800	175	1	9.0	.875
			10	90.0	8.75
			20	180.0	17.50
Halibut-liver Oil[1]	7300		1	36.5	
			10	365.0	
			20	730.0	
Carotene	7500		1	37.5	
			10	375.0	
			20	750.0	
Viosterol			1		50.0
(irradiated			10		500.0
ergosterol)		10,000	20		1000.0
Carotene plus	7500	1,000	1	37.5	5.0
Vitamin D[2]			10	375.0	50.0
			20	750.0	100.0

[1]I am grateful to Dr George Wald for supplying the halibut-liver oil and determining the U.S.P. units of vitamin A.

[2]Carotene plus vitamin D concentrate of cod-liver oil in cotton-seed oil was obtained from the SMA Corporation, Cleveland, Ohio.

General Description

The modifications observed in embryos injected with the preparations listed above are mainly in the vascular and nervous systems and in the rate of development. That these modifications are not attributable to the manipulations per se, is amply demonstrated by the normal behavior of the controls. Typical effects are indicated in Table 2.

There is very little indication of a specific response to any of the substances used. The effects produced in the embryo are typical in each of the categories observed. A few in each series, however, show no modifications in development, being similar to the controls. This variation is probably correlated with the "physiological state" of the embryo at the time of injection as influenced, for example, by seasonal variations in the vitamin content of the hen's diet and hence that of the egg. This point will be considered below. Delayed development is one of the typical responses of the embryo. In the retarded group there are two types; those which show a marked delay in development

TABLE 2.

	Typical Effects Observed
Rate of Development	Embryonic axis shorter than normal; cardiac and cranial flexures faintly indicated or completely absent; vitelline blood vessels not well developed or lacking; mesoblastic somites fewer than normal or poorly developed; diameter of the blastoderm less than normal.
Distribution of Blood	Clots in anterior and posterior cardinal veins; larger clots in terminal sinus and vitelline vessels.
Heart Rate	Slower than that of the control.
Development of Heart	Straight without sigmoid curve, amorphous mass, ventricle fused with sinus venosus.
Development of Blood Vessels	Anastomosis of adjacent vitelline vessels; poorly developed vessels (extra- and intra-embryonic); large blood islands, indicating a delay in the differentiation of blood vessels.
Central Nervous System	Absence of cranial and cervical flexures; brain flexures exaggerated; omphalocephaly; reduced brain.
Embryonic Membranes	Absence of amnion, chorion, and allantois (72-hour); amnionic head-fold poorly developed; tail-fold absent; amnionic folds fail to concresce.

but are otherwise similar to controls of an earlier stage, and those which, in addition to delayed development, show structural abnormalities. The two types are not separated in Table I. In many, the embryonic axis is straight without indication of the cranial and cervical flexures. The most pronounced effect is upon the vascular areas. In extreme cases, blood vessels have failed to develop and the vascular area lacks the characteristic pattern. Other embryos show less extensive abnormalities. In these, the vascular pattern is modified by intravenous and arterio-venous fusion. Blood islands are still numerous throughout, being more prominent in the posterior and lateral regions.

The blood in some is similar in color to that of the controls, although abnormalities are present in other regions. In others, very pale blood is observed, apparently because of deficiencies either in hemoglobin or in the number of erythrocytes or in both. The abnormalities of the heart include a reduction in its size, its malformation, and the extent and rate of pulsation. In some cases the heart is very large; in others it is markedly small, lacking the characteristic curvature. The pulsation of the heart differs from that of the control both in respect to frequency and to extent. Congestion of blood was oc-

casionally observed in the vessels of the head, but more often in the marginal sinus of the vascular area. These congested areas are gorged with blood and are easily detected without the aid of the microscope. Such gorged areas are not observed in embryos of the above mentioned "pale" type. Changes in the intensity of the color of the blood are least frequently observed where liver extract is used. In a few instances omphalocephalic and platyneurian embryos are found. The latter effect is found most frequently in embryos which have been treated with the vitamin D substance.

Typical effects upon the nervous system are indicated by the absence of flexures in the brain, reduction in brain size, and by the incomplete closure of the neural tube. In a few cases the cervical flexure is exaggerated, extending posteriorly to the level of the yolk stalk. Due to the fusion of the heart primordia above the brain, in some the brain is flattened and distorted (omphalocephalic). Frequently, the spinal cord is malformed and shorter than normal, and the embryonic membranes are either poorly developed or absent.

Table 1 shows a summary of the frequencies with which the effects appear in embryos after injections. With the two exceptions mentioned above and the one to be cited below, there is no clear evidence that the response of the embryo to vitamin A is different in kind from that to vitamin D. Nor does there seem to be a specific response to these vitamins when combined (cod-liver oil, halibut-liver oil, and vitamin D plus carotene). The effects upon the embryos of each group are similar.

Interrelationships between responses are indicated by the frequency with which they occur. Although some correlation between responses can be observed in many cases, no effect is regularly accompanied by others. For example, retarded development is not in each instance accompanied by congested blood vessels, nor is a congestion of blood in the terminal sinus always associated with gorged cephalic vessels. As might be anticipated, however, congestion in the terminal sinus and abnormal pulse rate of the heart occur with such similar frequency, that a causal relationship is suggested. As stated above pale blood is found less frequently in embryos treated with liver extract than in other classes.

TABLE 3

TABULAR SUMMARY OF EFFECTS IN 48 AND 72-HOUR EMBRYOS AFTER INJECTIONS OF 1, 10, AND 20 DROPS OF THE VARIOUS SUBSTANCES.

No.	Dr.	Normal		Retarded		Hemorrhage Head		Mg.Sin.		Abn. Hrt. Rate		Abn. Hrt.		Pale Bl.		Abn. Bl. Ves.		Abn. C.S.N.		Dead		No. Used	
		48	72	48	72	48	72	48	72	48	72	48	72	48	72	48	72	48	72	48	72	48	72
CAROTENE IN OIL																							
1		7	3	7	7	—	1	7	5	5	4	2	1	6	6	5	3	2	—	4	4	18	14
10		3	5	7	5	—	—	6	6	4	6	5	1	5	7	7	3	2	—	4	2	14	14
20		2	3	7	6	1	2	3	7	4	6	3	2	5	5	5	6	3c	2	8	6	15	16
COD-LIVER OIL																							
1		11	7	6	8	—	—	6	7	6	4	3	6	5	2	5	4	2	2	1	1	18	16
10		4	6	11	7	3	2	6	2	6	3	5	4	3	3	6	4	4	—	3	6	18	19
20		2	5	10	8	—	—	3	3	2	3	3	5	3	2	2	—	—	—	4	5	16	18
HALIBUT-LIVER OIL																							
1		3	2	2	3	—	—	2	1	—	—	2	—	2	—	2	—	1	—	—	2	5	7
10		1	3	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	4	4
20		—	1	2	—	—	—	2	3	2	3	—	3	—	3	—	2	—	—	2	1	4	5
CAROTENE PLUS VIOSTEROL																							
1		4	3	6	7	—	1	5	5	3	5	2	3	5	7	5	7	2	3	1	1	11	11
10		3	3	5	4	—	—	5	3	5	4	5	4	5	3	5	4	4	3	5	5	13	12
20		3	1	1	2	—	—	1	2	1	2	1	2	4	2	1	—	1	2	8	7	12	10
VIOSTEROL																							
1		4	4	8	8	1	—	4	6	5	2	5	4	7	5	7	6	5	4	3	—	15	12
10		3	—	4	6	—	—	3	4	3	5	1	5	4	5	5	5	2	3	5	4	12	10
20		2	2	1	4	—	—	1	4	1	4	—	3	1	4	—	2	—	4	3	4	6	10
LIVER EXTRACT																							
1		6	6	4	4	—	—	4	7	4	6	4	3	1	4	3	3	3	3	—	4	10	17
10		6	5	5	3	—	—	4	2	4	2	—	2	3	2	3	1	2	2	—	4	11	12
20		6	19	3	5	—	1	2	5	—	3	—	2	—	5	3	—	—	—	—	4	9	28
LIVER EXTRACT PLUS CAROTENE																							
1		3	5	2	4	—	—	2	2	—	—	—	—	—	—	—	—	1	—	—	—	5	9
10		2	1	3	2	—	1	3	2	1	2	1	3	—	2	1	2	—	1	—	1	5	4
20		—	8	10	11	1	—	8	18	5	13	3	5	2	5	1	8	2	1	1	8	11	34
COTTON-SEED OIL																							
1		14	13	2	2	—	—	—	2	—	—	—	—	—	—	—	—	—	—	1	1	16	16
10		10	7	—	5	—	—	—	2	—	4	—	—	—	—	—	—	—	—	5	2	15	14
20		10	15	3	2	—	—	—	1	—	2	—	2	1	3	2	2	—	1	3	9	16	26
NUJOL																							
1		5	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	6	6
10		4	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	6	6
20		2	2	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2	7	5

LIVER EXTRACT

Because of the pronounced effect upon the vascular picture at these stages, an early influence upon erythropoiesis was indicated. After checking with liver extract,[1] however, it is observed that there is no significant difference in the nature of the response. The erythrocyte count of the general circulation is not accelerated. These results are similar to those reported by Hays, Last, and Koch (1942). These

[1]The liver extract (intravenous, no. 227) was prepared by Parke, Davis & Co.

workers made injections of liver extract into the egg prior to incubation and found no quantitatively specific response in the rate of maturation or in the formation of erythroid elements in the developing embryo.

Table 2, which shows various seasonal relationships, however, indicates that where liver extract is used, there is a much greater number of normal embryos during the winter months than at other seasons. Embryos treated with liver extract show effects which are similar to those produced by substances of known vitamin content or their precursors. According to Salmon and Guerrant (1931), liver extract is an excellent source of vitamin G(B_2). It seems evident that since this vitamin is already present in the egg, an increase in the supply available to the embryo brings about embryonic changes in development which are similar to those caused by increases of vitamins A and D.

It is apparent that when the data obtained are reviewed with respect to the season of the year at which the experiments were made, that there is a correlation between the season and the percentage of normal embryos obtained following injections of the vitamin-containing preparations. Thus the injection of substances containing vitamin A or its precursors yield a significantly higher percentage of normal embryos when the injections were made during the winter months than did the same injections made during the spring and summer months. The data for liver extract also indicate a similar seasonal variation in the effects on normality of development. No similar correlation between the frequency of deaths and season are observed.

Aside from the differential absorption of the injected material resulting from the chance placement of it in relation to the position of the blastoderm, the relative concentration of the substance used may vary depending upon the amount of it already present in the egg. It has been demonstrated that the vitamin-content of the egg varies with the maturity of the hen laying the egg and with the vitamin-content of her food supply. Both of these factors may profoundly affect the responses of the developing embryo to unbalanced or toxic concentrations of the substances employed. Thus the embryonic system can be shown to vary in its responses according to the season in which the egg has been produced, and the result can be seen in the relative number of normal individuals obtained from the treated eggs.

That seasonal variations may involve changes in the fat-soluble fraction of the yolk of the hen's egg has been shown by Bethke, Ken-

TABLE 4
SEASONAL VARIATIONS

		Fall	%	Winter	%	Spring	%	Summer	%
Cod-liver oil	No. Injected	18		87		—		—	
	No. Normal	3	16	32	36	—		—	
Carotene in oil	No. Injected	7		35		—		49	
	No. Normal	—		19	54	—		4	8
Viosterol	No. Injected	—		—		34		31	
	No. Normal	—		—		8	23	7	22
Halibut-liver oil	No. Injected	—		—		29		—	
	No. Normal	—		—		10	34	—	
Carotene + vitamin D	No. Injected	18		29		—		22	
	No. Normal	2	11	15	51	—		—	
Liver extract	No. Injected	20		53		14		—	
	No. Normal	6	30	35	66	7	50	—	
Liver extr. + carotene	No. Injected	19		9		40		—	
	No. Normal	6	31	5	55	8	20	—	

nard, and Sassaman (1927). They found that the amount of the fat-soluble substances in the egg is greatly influenced by the diet of the hen and by her environment. Thus, eggs of confined hens showed 1/5 the potency in vitamin A and 1/10 the antirachitic value of those of hens fed on the same basal diet but with access to a blue grass range. If the yolk of summer eggs has a much higher content of vitamin A or its precursor than does that of winter eggs, it is evident that the excess of vitamin or its precursor injected into the yolk will have less chance of raising the total content above the defect threshold in the winter eggs than in those produced during the spring and summer.

DISCUSSION

In this study some of the vitamins present in the hen's egg, and hence available to the embryo, were increased by direct injection into the egg. The method employed is a widely used one and the control experiments leave little doubt that the mechanical disturbances which cannot be avoided, are only to a slight degree associated with the effects observed. It is assumed then, that the observed effects are due to the increased vitamin content of the embryonic diet. Since the bird's egg contains all of the vitamins except vitamin C Surrendre (1934), and Needham (1942), an excess of those injected into the egg is pro-

duced, thus causing a vitamin imbalance. The data presented here indicate that such an imbalance of vitamins is toxic to the developing embryo.

The toxic effects of an imbalance of vitamins has been demonstrated for rats by Harris and Moore (1928), by Hopkins (1923), and by Richardson, Palmer and Kennedy (1928), and for rabbits by Norris and Church (1930). Browning (1931) in summarizing the available data on vitamin imbalance, concludes that a vitamin imbalance, induced by experimental hypervitaminosis A and D, has a definite toxic effect as pronounced as a partial deficiency of all, or a complete absence of one. In the studies described above we may also be dealing with the effects of a vitamin imbalance. The additions to the egg of one or more vitamins or their precursors may cause an imbalance of vitamins which is toxic, and this toxicity is manifested in the abnormal development and differentiation of the embryo.

Vitamins A and D when used separately and to-gether, have similar effects upon the embryo, though there is a more frequent disturbance in the nervous system when the D active substance is used. Specific relationships between the vitamin and the effect upon the embryo is not established, nor is there a significant quantitative relationship. The changes in the vascular pattern, however, are more pronounced than those upon other systems. Since this is true, the differentiation of other regions would also deviate from the normal, as their normal development depends upon the utilization of food material made available by the vascular system. Probably greater and more specific effects would obtain where the vascular system is least or the last to be disturbed, thus insuring transportation of the injected materials through the various regions of the embryo.

Quantitative relationships could not be established, since embryos treated with one drop of the vitamin-containing substances showed effects as pronounced as those treated with 10 and 20 drops of the same substance; while others, although treated with larger amounts showed no effects. In this latter group, failure of the embryo to respond is probably due to a difference in the rate and in the extent of incorporation of the injected materials, and also to the "physiological state" of the embryo at the time of injection. Preliminary studies on the cytological effects of hypervitaminosis upon the embryo, indicate that this is a more delicate method of determining specific

and quantitative relationships. The study reported here is suggestive in that a disturbance in the vitamin balance of the hen's egg is brought about when hypervitaminosis is induced, and that this vitamin imbalance has a toxic effect upon the developing embryo. This toxicity is manifested in the appearance of abnormal structural and accompanying physiological effects which are as pronounced as those manifested in deficiency diseases of the growing chick.

Summary

1. Varying quantities of vitamins A and D or their precursors were injected into the hen's egg of 24 hours incubation.
2. The addition of these vitamins or their precursors upsets the vitamin balance of the embryonic diet.
3. This vitamin imbalance has a toxic effect upon the embryo which causes a delay in its development, and abnormalities of the nervous and vascular systems.
4. There is no significant correlation between the vitamin employed and the specific effect produced, nor was a quantitative relationship observed.
5. The failure of some embryos to respond to the increase of vitamins may be due to differential incorporation of the vitamins and also to seasonal variations in the vitamin content of the egg.
7. Liver extract has toxic effects upon the embryo which are similar to those caused by vitamins A and D.

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THE SELECTION OF CHICKS FOR GROWTH EXPERIMENTS AND THE EVALUATION OF GROWTH

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Introduction

In studying the effect of nutritional change on growth an effort is always made to reduce normal variation in response. This may be attempted by selecting the animals that are to be studied with the aim of eliminating those most likely to show aberrant behavior or by increasing the number of animals in a group or by averaging the results in one way or another to obtain representative values. This last is usually done by algebraic (statistical) methods. It is the purpose of the present paper to present a new method for the selection of animals and other methods which appear to be at least rare in the field of nutrition for the evaluation of growth (1). These methods, which are purely graphical give results that are less variable than those usually obtained with groups of the same size and are mathematically simpler and less time consuming than algebraic (statistical) procedures.

While these methods were worked out with chicks, they should be generally applicable to small laboratory animals. They are primarily designed for cases in which the reaction to change in the diet is rapid, as for example with amino acids or chlorine. Briefly, the methods are as follows: The newly-hatched birds are raised on a conditioning or pre-test diet for three weeks. Birds are then selected on the basis of their growth during the third week and their weight at the end of the third week. There is thus a double criterion for selection; absolute weight and rate of growth. The latter does not appear to have been used before, but it is obviously important and its importance increases with the shortness of the test period. The selected birds are placed on the test diet on the twenty-first day and weighed daily for a week. The experimental growth, expressed as the daily percentage increase

in average weight, is determined from the difference in ordinates of the best straight line (determined graphically) through the daily weights plotted against time. The use of a straight line rather than the difference in final and initial weights commonly used tends to eliminate the effect of erratic and meaningless day-to-day fluctuation. Finally, the experimentally measured growths are adjusted to that which a bird of standard weight would have, by means of a predetermined regression coefficient. The regression coefficient while common in other fields does not appear to have been sufficiently emphasized in nutritional studies. It furnishes a means of eliminating the effect of the normal change of growth rate with absolute weight. The third and fourth weeks were chosen for the selection of birds and measurement of growth because graphical examination of the growth data on a hatch of forty single-comb White Leghorns raised on a practical rearing diet in this laboratory several years ago showed little correlation between growth in the second and third weeks, but better correlation between growth in the third and fourth weeks. Furthermore, during this period, growth is nearly linear (2). The short period (a week) used to determine growth has several advantages: (1) the birds do not have time to depart markedly from the growth rates they showed in the third week which values entered into their selection; (2) secondary complicating effects of a primary deficiency have little time to appear; (3) the use of costly food components is reduced to a minimum.

The methods themselves will be described first, and then the results of applying them to a group of 158 single-comb White Leghorn chicks (hatch no. 6) will be described.

Selection of Birds

Each bird is weighed on the fourteenth and twentieth days and its gain in weight plotted against its weight on the twentieth day. This gives an array of points the long axis of which is determined by inspection. A band is marked off with sides parallel to this axis which includes the closely grouped points, but excludes all those showing either very small or very large final weights, or weight gains that are large or small compared to the final weights. The band thus established is divided into ten transverse blocks each containing the same number of points. (Figure 1 is a reproduction of the work sheet used

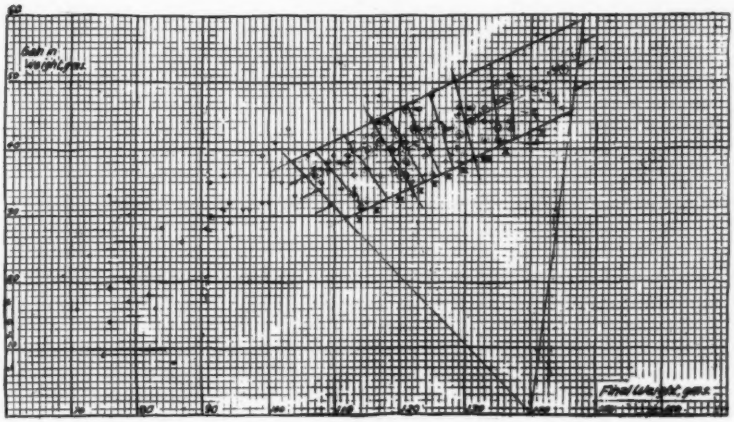

FIGURE 1
THE SELECTION OF CHICKS FOR GROWTH EXPERIMENTS AND THE EVALUATION OF GROWTH
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in making selections from the 158 chicks that are considered later). One bird from each group thus established is selected to form the material for a single growth experiment. Thus at the beginning of the test period the birds in any pen form a series showing both increasing absolute weight and increasing rate of gain in weight. And each bird in each pen has companions in all the other pens showing close to the same absolute weight and rate of gain. Thus the average weight and rate of gain is close to the same in all the pens.

MEASUREMENT OF GROWTH IN THE TEST PERIOD

The selected birds are weighed daily, at the same time of day, from the twenty-first to the twenty-eighth day, but in evaluating growth the twenty-first day itself is not considered because the change in diet and moving of the birds often causes a temporary departure from what subsequently turns out to be normal growth. The daily weights of each bird from the twenty-second to the twenty-eighth day are plotted against time, and the best straight line, determined by inspection, is drawn through them. In drawing this line, it is kept in mind that what is wanted is a measure of the normal reaction to metabolic causes uncomplicated by chance variation. For example, if a bird

fails to eat on one day, and so a single point falls very much below the trend established by the other points, it is disregarded in drawing the line. (Figure 2 reproduces an actual work sheet in which the diet used was quite good. With poorer diets, the departure of points from the straight lines may be greater).

Discussion

(1) *The regression coefficient.*

If the growths of the birds in a pen are plotted against the weights they had at the beginning of the test period, it will be found that growth increases as the initial weight decreases. Graphical examination of about 200 birds fed slightly different diets during the 4th week of

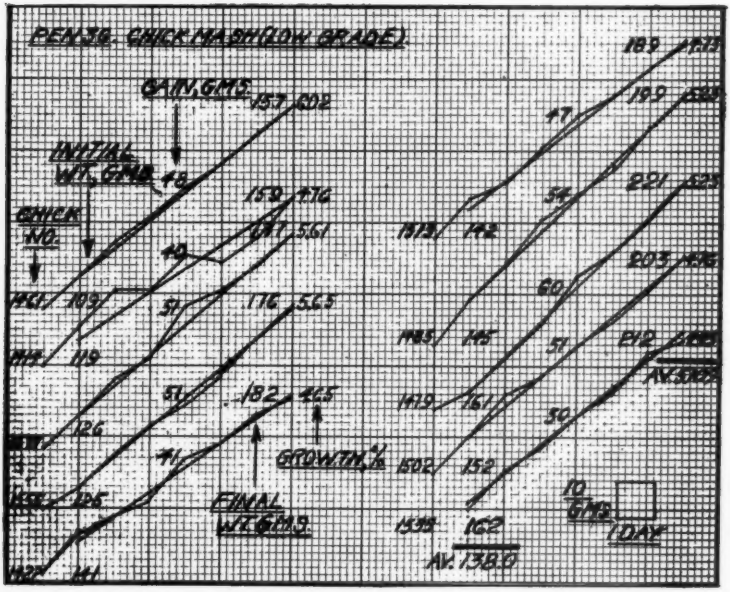

FIGURE 2

The Selection of Chicks for Growth Experiments and the Evaluation of Growth

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life, which gave growths of from 65-100% of normal, gave a regression coefficient of 0.0175% per gram increase in initial weight. The existence of this relation between growth and weight (4) shows that the greatest precision that growth data are capable of yielding cannot be got if birds or groups of birds of differing initial weights are compared. But it also shows that growth data can be adjusted by means of such a regression coefficient to a standard initial weight at which comparison may best be made. Since over the range of initial weights that occur, the relation of growth to weight is linear, or practically so, the standard initial weight of 100 grams has been used, though the average initial weight of 22-day-old birds that have been fed an adequate diet is about 125 grams.

In evaluating the growth of a pen of ten birds the actual growths obtained from the straight lines are plotted against the initial weights. (see Fig. 3 which is a reproduction of the work sheet used in adjusting the growths shown in Fig. 2). Through the point corresponding to the average growth and average initial weight a line is drawn with the slope of the regression coefficient. Experience has shown that any

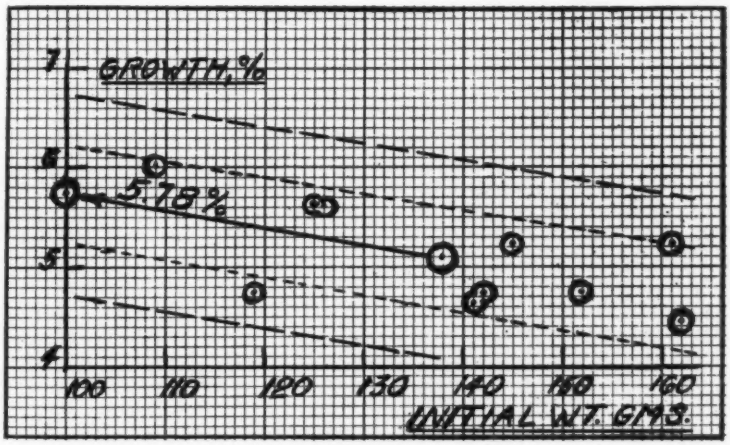

FIGURE 3
THE SELECTION OF CHICKS FOR GROWTH EXPERIMENTS AND THE EVALUATION OF GROWTH
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point whose growth departs more than one per cent from this line is best discarded and the average of the others (adjusted to the standard initial weight of 100 grams) used to determine the growth representative of the pen. The discarded points, which have been found to average less than one in a pen of ten birds, usually depart considerably more than one per cent from the mean line and represent birds to which something exceptional has occurred.

(2) *Application of the methods.*

To test the limitations of the above graphical procedures they were applied to a hatch of 158 birds that was fed a practical rearing diet during the entire period. This diet contained (per cent) ground grains (50), wheat bran (15), heated soybean meal (10.5), alfalfa meal (7.5), fishmeal (5.5), sardine oil containing 1000 units of vitamin A and 400 units of vitamin D per gram (0.25). Selection was made at the end of the third week, as described above, and the 110 selected birds grouped 10 in a pen. The 48 culled birds were also grouped in pens for later comparison of their performance with the selected birds. The growths in the eleven selected pens were evaluated as described above. In the final evaluation of the growths, eight birds from the eleven pens were discarded because of aberrant behavior according to the rules laid down in the description of the method. The results obtained are shown in Table 1. The regression coefficient used

TABLE 1

Pen No.	Avg. initial weight	Exp. growth	Growth adj. to initial weight 100 grams	No. of birds used
1	137.4	6.10	6.75	10
2	137.1	6.12	6.77	9
3	139.8	6.07	6.77	10
4	140.9	6.05	6.77	8
5	137.3	6.19	6.84	10
6	139.3	6.09	6.78	10
7	143.5	6.06	6.82	8
8	137.5	6.06	6.71	8
9	141.0	6.16	6.87	9
10	133.8	6.17	6.76	10
11	137.8	6.25	6.84	10
			6.79	102

was 0.0175% growth per gram change in initial weight. As an empirical test the result is quite satisfactory; none of the pens vary as

much as 0.10% from the mean of the eleven pens. It may be added that this method has been applied to over a thousand birds fed numerous diets and in no case have duplicate pens varied more than this amount.

(3) *Statistical evaluation of the results.*

In order to test the reproducibility of the regression coefficient used above, which was obtained by graphical analyses of the growths of about 200 birds, fed somewhat inadequate diets, the growths of the entire 158 birds were plotted against their initial weights and the regression coefficient then calculated, using the formula,

$$a = \frac{n\Sigma xy - \Sigma x \Sigma y}{n\Sigma y^2 - (\Sigma y)^2}$$

Seven points of very low initial weight and growth which obviously represent birds to which something untoward has occurred were omitted from the calculation. About the straight line thus determined a band was drawn to include all points less than 1½% in growth from the line. Seven points fell outside this band and, discarding these, the line was recalculated. The process was then repeated, narrowing the band to ±1%. This process of approximation was repeated until successive approximations produced no more change in the number of points included within the band. The last three approximations gave regression coefficients of 0.0169, 0.0160, and 0.0168% and so agree quite well with the value of 0.0175% previously established on other birds, fed other diets, by graphical means. With birds of an average weight of 120-140 gms a difference in regression coefficients of 0.0168% and 0.0175% would produce only about 0.01-0.03% of difference in the growth adjusted to an initial weight of 100 gm. Indeed the absolute value of the regression coefficient is of relatively little importance since, as explained already, the birds are so selected that the range of initial weights and the average initial weights of the pens are approximately constant. Changes in the regression coefficient will then produce little effect on the relative growth of pens, but change only the absolute values.

Twenty-nine points altogether were omitted from the calculation of the regression coefficient. The value of the double criterion used in the pre-test selection of birds is seen in the fact that sixteen of

these rejected birds came from the forty-eight culls and only thirteen from the 110 selected birds, and also in that the standard deviation from the final straight line of the culled birds used in the calculation was $\pm 0.52\%$, and of the selected birds was $\pm 0.31\%$.

The question now arises whether ten birds in a pen is a sufficiently large number to reduce the variance between pens to a negligible amount. Empirically, the results obtained on the eleven pens in Table I indicate that it is. The variance for the 102 birds finally used in determining the growths has, however, been calculated, using the formula:

$$\Sigma y^2 - \frac{(\Sigma y)^2}{n} = \text{sum of squares (total)}$$

$$\frac{(\Sigma y_1)^2}{n_1} \cdots\cdots \frac{(\Sigma y_{xI})^2}{n_{xI}} - \frac{(\Sigma y)^2}{n} =$$

sums of squares within pens.

Where y is the adjusted growth of a single bird and n the number of birds. In this calculation the 48 birds discarded during the pretest period are of course omitted since these are birds that would not be used in growth experiments. The eight birds discarded in evaluating the growth of the 11 pens are also discarded. It is only the variance of the birds that succeeded in passing the criteria of selection and performance set up that is desired. If these 8 birds were included the calculation would have no significance as a measure of the degree of reproducibility that the method yields. The results are shown below:

Variance	Degrees of Freedom ie n-1	Sum of Squares	Standard Deviation	Standard Error
Total	101	10.39	0.312%	0.0308%
Between pens	10	0.22	0.148%	0.0446%
Within pens	91	10.17	0.324%	0.0341%

The fact that the standard deviation between pens is smaller than the standard deviation within pens shows that the method of selecting birds and evaluation growth reduces the variance between pens to negligibility. The standard error between any two pens is 0.14%

(from $0.324 \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$.)

Consideration of the standard deviation within pens shows that ten birds in a pen is a good compromise number, since reducing the number of five would increase the standard error between any two pens to $\pm 0.21\%$, and increasing it to twenty would only reduce the error to 0.10%. To reduce the standard error to 0.05% would require ninety birds.

As an illustration of the practical application of the methods, the growths of 3 pens of 10 birds each used in nutritional experiments on methionine and cystine and showing only small differences in growth are shown in the table below. The probability that these differences in growth are due to chance has been calculated using the formula:

$$\frac{Mx - My}{\sqrt{\frac{\Sigma\delta x^2 + \Sigma\delta y^2}{2(n-1)}}\sqrt{\frac{1}{n} + \frac{1}{n}}} = t$$

in which M is the mean adjusted growth of a pen, δ the deviations of the individual adjusted growths from the mean, n the number of birds in a pen and t the function which by comparison with the usual tables gives the probability. The result is shown in the last column of the table.

TABLE II

Hatch & Pen. No.	Initial Weight	Exp. growth	Adjusted growth	Pens compared	Diff. in growth	Probability
	gms	%	%		%	%
IV 4	134.8	4.83	5.37	VII 36/V 1	0.13	48
V 1	138.1	4.98	5.65	V 1/IV 4	0.28	14
VII 36	138.0	5.11	5.78	VII 36/IV 4	0.41	1

The validity of the rejection of 8 birds from the 110 birds selected for the evaluation of the growths in the 11 pens, according to the empirical rule laid down that they departed more than 1.00% from the means of the pens in which they occurred, may now be considered. The standard deviation of the 110 birds from the representative straight line calculated above was $\pm 0.31\%$. Using this value and Chauvenet's criterion (3) for the rejection of extreme variates the limiting deviation is found to be $\pm 0.88\%$. Retaining the 8 rejected birds would decrease the mean adjusted growth in the 11 pens from

6.79% to 6.68% so that all values below 5.80% should be discarded. The 8 points had adjusted growths of 5.68 (II), 5.00 and 5.59 (IV), 4.39 and 5.66 (VII), 5.51 and 5.05 (VIII) and 5.74 (IX) and so fall outside Chauvenet's limit.

Applying Chauvenet's criterion to the 158 points used in determining the regression coefficient above and using the standard deviation of the 158 points ($\pm 0.39\%$) justifies the rejection of all points varying more than $\pm 1.1\%$ from the mean line which in turn justifies the rejections that were made in the semi-graphical method used in determining the line.

To determine whether more rigid selection of birds in the third week would be of value, the band produced by plotting weight on the twentieth day against gain in weight from the fourteenth to the twentieth day was divided into four longitudinal ribbons, (see Fig. 1) and the average growth of the birds in each such array, for the test period, calculated. Counting the groups from the bottom up, the results were:

TABLE III

	Avg. growth adj. to initial weight 100 grams	Standard Deviation
1st group	6.39%	.33%
2nd group	6.67	.46
3rd group	6.80	.41
4th group	6.97	.36

The similarity in the standard deviations of the four ribbons shows that there is no advantage to be gained by narrowing the band and excluding more birds.

The average final weights (Fig. 1) of the birds in the four ribbons are nearly the same, but the gains in weight from the 14th to the 20th day increase. The increase in growth during the test period is therefore a further indication of the importance of selecting birds on the basis of weight gain as well as absolute weight. And this increasing growth in the groups during the fourth week as the growth in the third week increases also shows the importance, when selecting birds from successive hatches, of maintaining rigidly constant the limits used in selection, that is, the limits of the band shown in Figure 1.

Summary

A method of selecting chicks and of evaluating their growth has been developed such that in a pen of ten birds the standard error in determining the daily percentage increase in average weight during the fourth week of life is $\pm 0.1\%$.

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THE RELATION OF INBREEDING TO CALF MORTALITY

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Introduction

None of the inbreeding experiments with cattle has approached the detailed studies conducted with laboratory mammals—for example, the Work of King (1918a, 1918b, 1918c, 1919) with the rat, and of Wright (1922a, 1922b) with the guinea pig. Inbreeding studies with cattle fall into three general categories: (1) reports on herds closed to outside animals ("blood") for many years: (2) studies, from studbook records, of the amount and kind of inbreeding that has occurred in breeds; (3) actual inbreeding experiments. The studies under the first two classifications fail to give much specific information on the relation of inbreeding to fertility, vigor, mortality, and size. Studies under the third classification should provide the data, but relatively few attempts have been made to study inbreeding in cattle. Geneticists and breeders working with cattle have generally assumed that the inbreeding results obtained with laboratory mammals would apply in a general way to cattle.

Within recent years there have been a few reports on the effect of inbreeding in cattle, but either the numbers of animals involved were few or the degree of inbreeding was relatively low. Certain trends are nevertheless indicated. Woodward and Graves (1933) found that inbreeding—sire-daughter matings—decreased the birth and mature weights of grade Guernseys. In their work with Holsteins, inbreeding did not affect fertility, but birth weights and growth rates were reduced; they also observed higher mortality and more deformities caused by recessive combinations among the inbreds. In their recent study of Holsteins, Woodward and Graves (1946) presented evidence indicating that inbreeding adversely affects fertility, calf mortality,

[1]This study was supported by the Kellogg Fund of the University of California for the Application of Genetics to Farm Mammals.

birth weight, and size. Bartlett, Reece, and Mixner (1939) produced about 60 heifers with inbreeding coefficients (Wright's 1922C) ranging from over 0.05 to over 0.15. Birth weights were normal; and growth was normal at 5 and at 10 months of age.

Bartlett and Margolin (1944) studied body weight, height of withers, and heart girth of 67 outbred and 76 inbred heifers ranging in coefficient of inbreeding from over 0.05 to over 0.20. When all the inbred heifers were grouped together, body weight and heart girth at 2 years of age were significantly lower than in the outcrossed controls, but there was no appreciable difference in height. When the inbred heifers were segregated into classes, only the class of 0.20 and over manifested a significant decrease in weight, height, and heart girth at 2 years of age. Because their data on mature cows (5 years of age) are apparently selected, the over-all effect of inbreeding upon size is not too clear. Almost certainly, however, individual animals with an inbreeding coefficient of 0.20 or over are smaller than outcrossed controls. This finding was later confirmed by Margolin and Bartlett (1945). Kolesnik (1939) obtained data on 1,247 inbred calves of the Jaroslavl breed collected at the farm of the Jaroslavl district. Inbreeding caused body weight to become more variable. This effect is attributed to segregation and homozygosity. Calves of low birth weight showed greater mortality. Some bulls tended to sire calves with low birth weight; others, calves with high birth weight. Dickerson (1940) produced 71 Holstein calves with coefficients of inbreeding ranging from 0.125 to 0.25. He found that inbreeding tended to reduce the birth weight in both sexes. Six of 8 sires showed a weight reduction in their inbred progeny.

The relation of inbreeding to fertility, size, vigor, and mortality has not been adequately studied in cattle even though substantial progress has been made. Further, the numbers of animals involved and the degree of inbreeding employed have been too limited to give a comprehensive over-all picture of the effects of inbreeding. While this paper adds to the knowledge of inbreeding and its relation to calf mortality many unanswered problems still remain.

Purpose of Study

The purpose of this study is to analyze calf mortality in the University of California dairy-cattle-breeding experiment, which has been

in progress approximately 25 years. The study includes single births but does not include calves whose deaths are caused by previously known lethal factors, or by trauma resulting from physical force; nor abortions or stillbirths from Brucella positive dams. The primary purpose is to study the degree of inbreeding as it affects abortions, stillbirths, and post-natal mortality and, whenever possible, to compare the mortality of the progeny of different sires within the same inbreeding class. Other factors that cannot be ignored are the relation of mortality to sex, season, epidemics, feed, management, and medication. All inbreeding coefficients are calculated from the formula of Wright (1922C). The total numbers of calves involved in the study is 1,033, of which 775 are Jerseys and 258 are Holsteins.

Stocks

The stocks of Jerseys and Holsteins investigated are those used in the dairy-cattle-breeding experiment of the University of California. Other published reports concerning these stocks are those of Regan, Mead, and Gregory (1935, 1944); Mead, Gregory, and Regan (1942, 1943, 1946) and Gregory, Mead, and Regan (1942, 1943, 1945). Our most recent report (1946) describes the stocks adequately and may be consulted for details.

Sire-daughter matings were employed whenever possible; but since this type of mating can be only temporary, there are many irregularities in the mating systems used.

Care, Management, and Environment

Care, management, nutrition, sanitation, and medication must be considered in any study of calf mortality. Each was standardized and kept as uniform as possible from 1922 to 1944. Throughout this period, Jersey heifers were bred when they reached a weight of about 550 pounds, and Holsteins at a weight of 750, regardless of age. Early in the experiment, the Jersey heifers received limited amounts of concentrates; they did not reach breeding weight until about 18 months; calving at about 27 months. Beginning in 1935, in order to shorten the time between generations, heifers were fed liberal amounts of concentrates as well as hay after weaning; thus they reached breeding weight at 12 or 13 months of age, and calving at about 22 months. The method of feeding, management, and raising the Holstein heifers

has been the same throughout the experiment, being identical with that of the Jerseys before 1935. In general, cows were milked 305 days and were bred so that they had a dry period of approximately 2 months. During lactation they were fed a complete diet according to production. When dry they were put on pasture (if it was available) and were fed to regain any weight lost because of lactation. Lactating cows always received all the good alfalfa hay they would consume. When available, pasture was used to supplement the hay.

A few days before freshening, cows were brought to the barn. In good weather they were allowed to calve in clean corrals near by. In bad weather they were left in box stalls well bedded with fresh straw. The maternity stalls were kept sanitary. Here the animals were observed closely. Only occasionally did a cow freshen in the pasture. Cows that had no difficulty in delivery were left alone; but when needed, a veterinarian was in attendance. Immediately after birth, the herdsman made sure that the calf received colostrum milk. The calf was left with the cow for 2 days. The program was to weigh all calves soon after birth; assign a number to each one that was to remain in the herd and record all other pertinent data. Heifer calves twin to bulls were usually destroyed; all others were raised if possible. Since not all the bull calves could be used in the breeding experiment, many were destroyed. The sex and other essential data were recorded for all stillborn calves. Post-natal deaths and detected abortions were recorded, with as much of the essential information as could be obtained.

While with their dams, all calves nursed at will. Until 2 weeks old they received slightly less than 1 pound of whole milk daily for every 10 pounds of body weight given in three feedings—morning, noon, and night. Calves over 10 days old had roughage, concentrates, and water before them at all times. They were kept in individual stalls during their first 2 months. All received liquid skim and whole milk up to 90 days of age. From 3 to 6 months 18 per cent of dried skim-milk was added to the concentrate mix. After 1941, when dried skim-milk was difficult to obtain, an equal part (by weight) of fish meal was used to replace half of it. After 6 months of age, the dried skim-milk (or dried skim-milk and fish meal) was eliminated from the concentrate mix. Care and management henceforth have been described. The calves were always kept on a high plane of nutrition favorable to

growth and development, even though it was necessary to make changes in the concentrate mix, as by substituting the fish meal for a part of the dried skim-milk.

Every effort was made to keep the maternity stalls and calf barn clean and sanitary. After 1928 the herd was tested regularly for Brucella infection. Trichomoniasis was never found or suspected. Medication was administered by the Division of Veterinary Science of the University of California. Only one veterinarian looked after the herd at any one time, and medication varied with changes in the veterinary personnel and with developments in medical science. Sulfa drugs—after their discovery—were used regularly in the treatment of pneumonia and scours, two common diseases of young calves.

Selection of the Data

This study was confined to Jersey and Holstein calves born singly, fed and raised by the standard methods described. Twins were excluded because they manifested a much higher percentage of abortions, stillbirths, and deaths soon after birth. Cases of mortality caused by previously recognized specific recessive lethal genes were also excluded —for example, all calves afflicted with epitheliogenesis imperfecta (Regan, Mead, and Gregory, 1935) or recessive achondroplasia (Gregory, Mead, and Regan, 1942). Any other mortality attributable to specific recessive lethals that did not cause obvious morphological change was not identified before this study was begun. Such lethals may, however, have been segregating in the stock. Stillbirths or calves dying soon after birth as a result of dystocia, milk fever or other ailments which did not reflect the genetic vigor or viability of the individuals involved were also excluded.

All cases of accidental deaths after normal delivery are excluded. These included a few cases of drowning from falling into a watering trough or breaking the neck from struggling in a stanchion. None of these reflected the inherent viability of the individual. Admittedly, some accidental deaths may have been overlooked; but, if so, the number is negligible. A small percentage of those classed as stillbirths may actually have been accidental deaths; for example, a normal, vigorous, viable calf may have been smothered by the fetal membranes immediately after delivery. Usually, however, the herdsman was present to prevent such casualties. All stillbirths in which the dam was

free from *Brucella abortus* are included in the analysis. Except for cows known to be infected with *Brucella abortus*, all abortions are included, though possibly some of the abortions may have been caused by accidents or injuries suffered by the dam.

No calves on experimental diets were included in the study. An attempt was made to raise all heifer calves by the standard method described. Bull calves, however, not needed for breeding were destroyed at birth, a fact which accounts for the great differences in numbers of males and females in certain classes of inbreeding. In general, only males from cows with higher than average records of production were saved. This basis of choosing males to raise is assumed to be independent of any hereditary or environmental factors that condition viability during the first few months after birth. All details relating to the selection of calves used in this study are presented. If, by this selection, some bias is introduced (a possibility which the authors doubt), it is unintentional.

Abortions and Stillbirths

All the single births used in the study are summarized in table I. They are divided into four classes according to their coefficients of inbreeding (Wright 1922C). Class I includes all animals whose coefficient of inbreeding is from 0 to 0.1249; class II, from 0.125 to 0.2449; class III, from 0.245 to 0.3749; class IV, 0.375 and over. No inbreeding coefficient in class IV exceeds 0.47. Had there been more animals, it would have been desirable to break the coefficients down into class limits of 0.05. As it is, the classification fits rather well the mating system used, even though irregularities in the system were frequent. All the animals in class I resulted either from outcrossing or from mating animals not closely related. The matings that produced the animals of class II are rather irregular, but many were from sire-granddaughter matings. With very few exceptions, those in class III are of sire-daughter, often with other common relationships in addition. Almost all the animals in class IV are the result of sire-daughter-granddaughter matings, perhaps with additional common ancestry.

Over half the males, but none of the females, were destroyed at birth. The percentage of accidental deaths was about the same for males and females. The only reason for considering the animals de-

TABLE 1
SUMMARY OF ALL CALVES BORN SINGLY[1]
COEFFICIENT OF INBREEDING CLASSES

	0-0.1249 I	0.125-0.2449 II	0.245-0.3749 III	0.375 and over IV	Total
		JERSEY MALES			
Destroyed	92	24	56	14	
Accidental deaths	1	0	3	1	
Abortions	3	2	1	0	
Stillbirths	9	4	5	4	
Calves died before 4 mos. of age	11	5	21	13	
Calves lived	64	24	33	9	
Total	180	59	119	41	399
Abortions percentage	1.67	3.40	0.84	0.0	
Stillbirths percentage	5.00	6.78	4.20	10.00	
		JERSEY FEMALES			
Destroyed	0	0	0	0	
Accidental deaths	1	2	1	0	
Abortions	2	1	0	0	
Stillbirths	5	0	8	3	
Calves died before 4 mos. of age	19	22	26	16	
Calves lived	133	45	68	24	
Total	160	70	103	43	376
Abortions percentage	1.25	1.43	0.0	0.0	
Stillbirths percentage	3.13	0.0	7.77	6.98	
Total Jerseys					775
		HOLSTEIN MALES			
Destroyed	29	13	22	19	
Accidental deaths[1]	0	0	0	0	
Abortions	0	0	0	1	
Stillbirths	1	1	0	1	
Calves died before 4 mo. of age	0	0	5	0	
Calves lived	22	9	10	3	
Total	52	23	37	24	136
Abortions percentage	0.0	0.0	0.0	4.17	
Stillbirths Percentage	1.92	4.35	0.0	4.17	
		HOLSTEIN FEMALES			
Destroyed	0	0	0	0	
Accidental deaths	0	0	1	0	
Abortions	3	0	0	0	
Stillbirths	1	0	1	0	
Calves died before 4 mo. of age	2	2	7	2	
Calves lived	40	16	30	17	
Total	46	18	39	19	122
Abortions Percentage	6.52	0.0	0.0	0.0	
Stillbirths Percentage	2.17	0.0	2.56	0.0	
Total Holsteins					258
Total (Jerseys and Holsteins)					1033

[1]Brucella positive abortions and stillbirths, and sex undetermined abortions excluded.

stroyed and the accidental deaths is to obtain the percentage of abortions and stillbirths. There were relatively few abortions in either the Jerseys or the Holsteins, and the data indicate that the degree of inbreeding does not influence the percentage of abortions (table 1). There were about the same percentage of male and female abortions.

The percentage of stillbirths is greater than the percentage of abortions. Further, the percentage of stillbirths in the higher classes of inbreeding is greater than that of class I, the control group; but, according to X^2 tests, none of these increases over the control group are statistically significant. Probably the percentage of stillbirths increases with the degree of inbreeding; but the relatively small numbers are too limited to show a significant difference. The Jersey males and females manifest a greater percentage of stillbirths than the Holstein. A "t" test indicates that the difference in stillbirths between the breeds is significant at the 2 per cent level. On the whole, there were slightly more males stillborn than females; but the difference is not significant.

Total Mortality

Total mortality as used in this report includes all abortions, stillbirths, and deaths after birth up to 4 months of age. Two calves died from a peculiar liver derangement at 127 and 140 days of age, respectively. Since these two had identical symptoms with others that succumbed to the same malady at about 100 days of age, they are also included in the group; careful nursing probably kept them alive beyond the period of 4 months. Those excluded are calves that were destroyed at birth (only males); accidental deaths; all deaths caused by the lethal action of the genes conditioning achondroplasia, and imperfect epithelium; abortions and stillbirths from Brucella-positive cows; and—since the sexes are separated—a few cases of abortion or stillbirths from Brucella-negative cows, in which the sex of the calves involved was not recorded.

Table 2 summarizes the relation between the degree of inbreeding and the total mortality in females. Throughout this paper, the degree of inbreeding is usually indicated by classes that are represented by Roman numerals. All animals with an inbreeding coefficient from 0 up to 0.1249 were included in class I, since homogeneity tests indicated that within the class the calves having the higher coefficients of

TABLE 2
THE RELATION OF TOTAL MORTALITY TO THE DEGREE OF INBREEDING IN FEMALE CALVES TO 4 MONTHS OF AGE

Number of births	Coefficient of inbreeding (f) Class limits	Coefficient of inbreeding (f) Mean S.E.	Mortality Mean S.E.
	JERSEY		
159	0.0 — 0.1249(I)	0.03 ± 0.003	0.16 ± 0.029
68	0.125 — 0.2449(II)	0.19 ± 0.004	0.34 ± 0.057
101	0.245 — 0.3749(III)	0.28 ± 0.003	0.35 ± 0.047
44	0.375 and over[1] (IV)	0.41 ± 0.004	0.43 ± 0.075
	HOLSTEIN		
46	0.0 — 0.1249(I)	0.02 ± 0.005	0.13 ± 0.050
18	0.125 — 0.2449(II)	0.15 ± 0.004	0.11 ± 0.074
38	0.245 — 0.3749(III)	0.27 ± 0.005	0.21 ± 0.066
19	0.375 and over[2] (IV)	0.39 ± 0.005	0.10 ± 0.069

[1]Highest coefficent of inbreeding in the class is 0.47
[2]Highest coefficient of inbreeding in the class is 0.44

inbreeding showed no greater mortality than those having a coefficient of 0.

The mean coefficient of inbreeding of each class is given in table 2. The total mortality of classes II, III, and IV for Jersey calves is greater than that of class I, the control group, and in all comparisons with class I the difference is significant. The mortality of classes II and III is essentially the same. The mortality of class IV is substantially greater than that of classes II and III but probably because of the relatively small numbers the difference in either case is not statistically significant. Thus it is certain that in female Jersey calves the total mortality is positively correlated with the degree of inbreeding.

The data on Holstein females are more limited and somewhat inconclusive (table 2). The Holstein calves were less affected by inbreeding than were the Jerseys. The total mortality of class III is greater than that in either classes I, II, or IV. None of the differences in mortality between the four classes is significant, however. The high mortality in class III is probably caused by inbreeding. The low mortality in class IV is difficult to explain. The most tenable explanation is that sulfa-drug therapy reduced the death losses from pneumonia and scours. The highest percentage of the calves in class IV were born after this treatment was initiated. Since many of the bull calves were destroyed at birth, no analysis of total mortality was made of the Jersey and Holstein males.

Post-Natal Mortality

As was indicated in an earlier section, the percentage of abortions was not affected by the degree of inbreeding in either Jerseys or Holsteins. Although the difference was not statistically significant, the percentage of stillbirths did increase with the coefficient of inbreeding in Jersey males and females. Since most of the mortality occurred after birth, the greatest interest in the data is in the mortality of the calves born alive. With the exception of the two calves mentioned in an earlier section, all mortality considered is to 4 months of age. Obviously all abortions, stillbirths, accidental deaths, and calves destroyed at birth are excluded. Since many males were destroyed at birth, the females in the study greatly outnumber the males. The relation of the degree of inbreeding to post-natal mortality is shown in table 3. The mean degree of inbreeding for the different classes is essentially the same as for total mortality (compare with table 2). These data clearly show that with an increase in the coefficient of inbreeding in Jersey males, there is an increase in mortality after birth.

The post-natal mortality of Jersey females is in general agreement with that of the Jersey males. The mortality of the class I control group of females is the lowest among all the classes and, when compared with the mortality of the other three classes, is statistically significant. The mortality of class IV is the highest among all the classes but is not significantly greater than that of classes II and III. These results clearly show that in Jersey females an increase in the degree of inbreeding causes an increase in the mortality to 4 months of age.

The number of Holstein males was limited, and most were destroyed at birth. All the males of classes I, II, and IV that were selected for raising lived; but classes II and IV had only 9 and 3 calves, respectively. Of the 15 calves in class III, 5, or 0.33 ± 0.121, died. This suggests, at least, that the mortality of Holstein males increases with the coefficient of inbreeding.

The data on Holstein females, although limited, indicate that the degree of inbreeding may affect mortality up to 4 months of age. The control group of class I has a mean mortality of 0.05 ± 0.034. The mortality of all other classes of inbreeding is more than twice that of the control group but because of limited numbers none of the comparisons manifest statistically different mortality rates between the 4 classes of inbreeding. As will be shown in a later section, factors

of care, management, and medication also affect mortality; these factors are operating to increase the viability of the class IV Holstein females. Even though the Holsteins have less mortality than the Jerseys it seems safe to conclude that an inbreeding coefficient of 0.125, or greater, is attended by an increase in the mortality of Holstein calves.

Relation of Sex to Post-natal Mortality

The relation of sex to degree of inbreeding and post-natal mortality is of considerable theoretical import. In class II the females manifest a higher degree of mortality than the males, which is significant at the 5 per cent level. There is some doubt as to how this situation should be interpreted. The mortality of females in class II is greater than in class III; perhaps some uncontrolled factor or combination of factors may explain the exceedingly high mortality of the class II females. There is a higher mortality among males in inbreeding classes III and IV but the difference is not significant in either case. If classes III and IV are combined the mortality is just below the level of significance. It will take greater numbers to establish definitely the relation of degree of inbreeding to differential mortality between the sexes in the higher classes of inbreeding. The Jersey data justify the conclusion that there is no difference in mortality between males and females that have a coefficient of inbreeding of 0.1249 or less. Within the coefficient-of-inbreeding range from 0.125 to 0.2449, the relation of mortality to sex is not clear; but when all the data are considered, sex may not be a factor greatly influencing mortality. With coefficients of inbreeding of 0.25 and higher, males probably have a greater mortality than females. Since data on Holstein males are so few, no attempt is made to analyze differential mortality between males and females within the different inbred classes. The data available parallels rather well the results obtained with Jerseys.

If there is a differential mortality between the sexes in the higher classes of inbreeding, as is suspected, the possible causes are of interest. That sex-linked genes are involved is unlikely. To produce the coefficients of inbreeding in classes III and IV, sire-daughter matings were employed almost exclusively; thus any increased mortality in males from sex-linked genes would have to come from the dams. Dams heterozygous for a sex-linked semilethal or a gene conditioning

low viability should cause low viability in males produced in outcrosses. The low mortality in the class I males indicates that such genes are not involved. The differential mortality may be caused by sex-limited genes, but as yet there is no clear cut evidence that such genes are segregating in any of the populations. Another possibility is that males may, in general, have less viability than females; and when vigor is reduced by homozygous combinations, which are less viable, more males succumb than females.

The Relation of Breed to Post-natal Mortality

According to table 3, when sex and degree of inbreeding are taken into account, the Jersey calves consistently suffered a markedly greater mortality than did the Holsteins. This difference is consistent and is manifest in males and females for all classes of inbreeding. The reason is not clear. Since only two Holstein bulls were used and most

TABLE 3

The Relation of Post-Natal Mortality to the Degree of Inbreeding in Male and Female Calves

Number of live births	Inbreeding Coefficient (f) Class limits	Inbreeding Coefficient (f) Mean S.E.	Mortality Mean S.E.
	Jersey males		
84	0.0 — 0.1249(I)	0.02 ± 0.004	0.12 ± 0.035
31	0.125 — 0.2449(II)	0.17 ± 0.005	0.16 ± 0.066
55	0.245 — 0.3749(III)	0.27 ± 0.004	0.40 ± 0.066
21	0.375 and over[1] (IV)	0.39 ± 0.004	0.57 ± 0.108
	Jersey females		
152	0.0 — 0.1249(I)	0.03 ± 0.003	0.12 ± 0.027
67	0.125 — 0.2449(II)	0.19 ± 0.004	0.33 ± 0.057
93	0.255 — 0.3749(III)	0.28 ± 0.003	0.29 ± 0.047
41	0.375 and over[2] (IV)	0.41 ± 0.004	0.39 ± 0.076
	Holstein males		
22	0.0 — 0.1249(I)	0.02 ± 0.007	0.0
9	0.125 — 0.2449(II)	0.14 ± 0.005	0.0
15	0.25 — 0.3749(III)	0.26 ± 0.006	0.33 ± 0.121
3	0.375 and over[3] (IV)	0.40 ± 0.020	0.0
	Holstein females		
42	0.0 — 0.1249(I)	0.02 ± 0.005	0.05 ± 0.034
18	0.125 — 0.2449(II)	0.15 ± 0.004	0.11 ± 0.074
37	0.245 — 0.3749(III)	0.27 ± 0.005	0.19 ± 0.064
19	0.375 and over[3] (IV)	0.39 ± 0.005	0.11 ± 0.069

[1]Highest inbreeding coefficient 0.45
[2]Highest inbreeding coefficient 0.47
[3]Highest inbreeding coefficient 0.44

of the inbred progeny were sired by one of them, it is possible that the Holstein sample is not representative of the breed. When all the data are considered, however, the assumption that this differential mortality is dependent upon inherent breed characteristics seems tenable. It is known that Jerseys and Holsteins differ in many fundamental characteristics that have a genetic basis. The mean mature weights of the Jersey females is about 1050 pounds; that of the Holsteins 1300. The mature weights are reflected in the birthweights, the means being approximately 55 and 85 pounds for Jerseys and Holsteins, respectively. There are specific evidences of metabolic differences between the two breeds. The Jersey milk has a greater content of fat and solids-not-fat. Jersey milk contains more carotene than preformed vitamin A while the reverse is true of Holstein milk (Baumann, Steenboch, Beeson, and Rupel, 1934). The two breeds possess different upper critical body temperatures—80 and 85 degrees F for Holsteins and Jerseys, respectively (Regan and Richardson, 1938). Perhaps some of the different fundamental characteristics of the two breeds may account for the differential mortality found in the calves studied.

The Relation of Birth Weight to Post-natal Mortality

Birth weight as related to degree of inbreeding will be considered in another report concerning the effect of inbreeding upon growth and size. Since there may be a relation between birth weight and mortality on the one hand, and birth weight, degree of inbreeding, and mortality on the other, the relation of birth weight to mortality is presented here.

The analysis summarized in table 4 concerns only the calves that were born alive. These are segregated according to breed, sex, and degree of inbreeding. The mean birth weights, together with their variances, are listed for all the calves that lived and died within each inbreeding class. The discrepancy between the total numbers of calves that lived and those that died, as shown in table 4, and the numbers given in table 1 is explained by the fact that the birth weight of a few of the calves was not recorded.

Within a given sex and class of inbreeding, the Jersey calves that lived tended to have a slightly higher mean birth weight than those that died, though the difference is not significant in any of the comparisons. The only outstanding case in which the birth weight for the calves that died is heavier is in the class II males; but here, too,

TABLE 4
MEANS AND VARIANCES OF BIRTH WEIGHTS OF CALVES THAT LIVED AND DIED, ACCORDING TO DEGREE OF INBREEDING

F Class	Fate	Number of calves	Birth Weights lbs. Mean	Variance
		JERSEY MALES		
I	Lived	59	63.2	73.57
	Died	11	63.4	60.39
II	Lived	20	59.5	114.44[1]
	Died	5	66.2	7.20[1]
III	Lived	30	60.8	51.89[2]
	Died	19	58.8	128.53[2]
IV	Lived	8	60.1	12.82[1]
	Died	12	57.0	143.38[1]
		JERSEY FEMALES		
I	Lived	107	57.0	57.34[2]
	Died	16	52.4	126.59[2]
II	Lived	42	54.1	53.89
	Died	18	52.0	53.96
III	Lived	61	53.6	93.59
	Died	24	52.9	71.63
IV	Lived	21	56.3	49.44
	Died	14	56.0	59.54
		HOLSTEIN MALES		
I	Lived	21	79.6	74.46
	Died	0	——	——
II	Lived	7	80.1	58.47
	Died	0	——	——
III	Lived	9	84.3	49.78[1]
	Died	5	77.0	462.60[1]
IV	Lived	3	87.0	3.00
	Died	0	——	——
		HOLSTEIN FEMALES		
I	Lived	34	83.2	132.85
	Died	2	87.5	4.50
II	Lived	15	80.4	55.44
	Died	2	81.5	40.50
III	Lived	28	77.3	103.07
	Died	7	80.6	80.12
IV	Lived	17	71.5	94.15
	Died	1	53.0	——

[1]Difference in variance significant at 1 per cent level by Snedecor F. test.
[2]Difference in variance significant at 5 per cent level by Snedecor F. test.

the difference is not significant. Of the 5 calves that died, 4 were by the same sire; these will be considered later when differences in variances are discussed. Within a given sex and inbreeding class of Jerseys, therefore, when the progeny of all bulls are considered, the mean birth weights of the calves that lived and died are essentially the same.

Most bulls did not have enough male or female progeny within a given inbreeding class for individual progeny analysis of birth weights. Sires 300D and 370A, however, had enough female offspring in class I. The mean birth weight of 23 daughters of 300D is 51.3 ± 1.49 pounds; that of 42 daughters of 370A is 60.2 ± 1.05. The difference in birth weight between the progeny is highly significant. Thus it is evident that within the same sex and class of inbreeding, individual bulls sire progeny of a characteristic birth weight, which for convenience may be classed as light, medium, and heavy. These observations on the characteristic birth weights of the progenies of different sires agree with Kolesnik (1939). If mortality is positively correlated with low birth weight, the progeny of 300D should manifest a higher mortality than the progeny of 370A. The proportion of mortality for the 300D daughters is 0.13; for the 370A daughters, 0.12. The mean mortality of all the females in class I is 0.12. There is no evidence in the Jersey data that low birth weight, *per se*, is correlated with high mortality. The limited Holstein data are insufficient to show any relation that may exist between mean birth weight and mortality within the different classes of inbreeding.

Even though within a given inbreeding class the mean birth weights of calves that lived and of those that died are substantially the same, there is apparently some relation between birth weight and mortality. In several instances the variability in birth weight was greater for calves that died than for those that lived. Hence, the variances in birth weights within the different inbred classes of calves are compared (table 4). There are five instances in which the difference in variances of birth weight is significant at the 1 or 5 per cent level according to the F test of Snedecor (1940). Of these (with but one exception, which will be discussed last) the variance of the group that died is greater than that of the group that lived (Jersey males, classes III and IV, and females class I; Holstein males, class III). Although not all the calves that died are at the extremes in birth weight, those that possess the greater extremes, either light or heavy, manifest the greater mortality in the four classes under consideration. If the calves that are light or heavy at birth succumb more readily than those near the mean weight, then—if the numbers involved are few—one should encounter cases in which most of the calves that die are either light or heavy. Under such conditions, the variance in birth weight of the

calves that die would be significantly less than the variance of those that live. The class II Jersey males appear to be such an example. Here all the calves that died have a greater birth weight than the mean of those that lived. Incidentally, of the 5 which died, 4 were sired by one bull; these will be discussed in a later section in an analysis of individual sires.

Significant differences in variance of birth weights between surviving and non-surviving calves within the same inbreeding class and sex were observed in 4 of the 8 groups of Jerseys studied. Even though there are 8 groups of Holsteins, only the class III males and females are numerous enough to indicate the variations in birth weight as regards survival. Only in the class III males is the difference in variance significant. A study of 10 groups, 8 Jerseys and 2 Holsteins, shows that in 5 instances out of 10, the calves that lived differed significantly in variance of birth weight from those that died. Conceivably, when the progeny of several bulls are grouped together, as in table 4, differences in variation may be masked or accentuated if individual bulls tend to sire calves of low or high birth weight. As was shown in an earlier section, individual sires may produce progeny of a characteristic birth weight, light or heavy.

The Jersey sires, with few exceptions, produced too few progeny in any one class for an individual analysis. All the class III Holstein males, except one in the group that lived, were the progeny of one sire. When this single exception is removed, the difference in variance of birth weight remains significant at the 1 per cent level. Thus there is one clear-cut example of a difference in variance of birth weight between surviving and non-surviving calves within one class of inbreeding among the progeny of one sire.

Since calves manifesting extremes in birth weights are on the whole less viable than those of average weight, the cause for differences in variance in birth weight between calves that lived and died may offer a clue to the cause of mortality. Environmental or genetic factors may be operating to cause differences in weight at birth. Even though the feeding regime, care, and management were well standardized, variations could come from these sources. Other environmental factors that might be operating are age of dam, milk yield during the lactation before calving, length of rest period before calving, and general health of the dam. It may be possible to test the effect of some of the more

tangible environmental factors upon birth weight, but it would be difficult, if not impossible, to test the more intangible ones. In the absence of experimental evidence, it is assumed that most environmental factors that could influence birth weight may affect viability very little except in cases where the weight of the calf is greatly reduced. Since all the environmental factors were reasonably well controlled throughout the period, it is unlikely that variations in environment could be responsible for the extremes in variance in birth weights and the associated mortality. Krasnov and Pak (1939) reported that birth weight appears to be little influenced by environmental agencies such as time of mating, and length of dry period. Length of gestation, however, is positively correlated with birth weight.

It was shown earlier that within a given class of inbreeding and sex, the birth weight of the offspring of different bulls is significantly different. These differences can most logically be attributed to heredity. For this reason, most of the extremes in variation of birth weight were attributed—in the present study—to heredity. Most of the variation in birth weight within the progeny of a given sire is attributed to the segregation of definite genes or gene combinations, and in general the extreme segregates appear to be less viable.

Relation of Post-natal Environment to Mortality

The term *environment* as used in this report refers to the aggregate of all the external conditions and influences affecting the life and mortality of the calves. Specific factors considered are care, management, nutrition, medication, epidemics, and season. The feeding and handling of all the calves have been closely supervised by one man for over 20 years. Even though care, management, and nutrition were well standardized, it is humanly impossible to maintain identical conditions for each individual, but for practical purposes, at least, they are assumed to have been the same for all calves. Such factors as medication, epidemics, and season could not be controlled. Medication depended upon progress in medical science and was modified as new drugs were developed or superior methods of therapeusis were established.

Within a sex and class of inbreeding, homogeneity tests definitely indicated that month or season of birth did not affect mortality. If epidemics were a factor, they were probably of short duration. Their

occurrence was not indicated by the statistical tests; yet some of the mortality may have been epidemic.

Throughout the Jersey data, the percentage of mortality within a sex and inbreeding class was slightly higher in calves born before 1936 than in those born subsequently, but the difference was not statistically significant in any class. Since the results were consistent, it was suspected that there may have been a real difference before and after 1936; lack of significance may have been due to limited numbers coupled with only a slight difference. There was a complete change of Jersey sires during 1936, and any change in mortality could have been caused by the replacement sires. Since a difference in mortality before and after 1936 was suspected, additional tests were made to determine whether or not a change had occurred. Holstein sire 100B produced enough females within inbreeding class III before and after 1936 to provide an answer. Of the 12 calves born before 1936, 7 died, whereas all of the 21 born after 1936 survived. Chi square tests reveal that the difference in mortality before and after 1936 is statistically significant. This differential mortality is attributed to a difference in environment.

Since environmental factors were more conducive to viability in Holsteins after 1936 than before, attention is again directed to the relation of degree of inbreeding to mortality in Holsteins (table 3). All the calves, both males and females, in the class IV inbreeding group were sired by 100B, and all were born after 1936. With the exception of one calf that lived, all the Holstein males in class III were sired by 100B. Most of these calves were born after 1936, but two that died had birth dates before that year. Evidently more favorable environment after 1936 accounts for the low mortality in the higher inbreeding classes of the Holsteins. Even though it cannot be demonstrated, it is assumed that whatever differences in environmental factors were affecting mortality in the Holsteins before and after 1936 were also operating in the Jerseys. The possible differences in environmental factors before and after 1936 will now be considered.

As was mentioned previously, beginning in 1936, the calves were given additional concentrates to make possible reproduction at an earlier age. The increase in the feeding of concentrates does not begin until after 4 months of age, and therefore should not affect the mortality. There were improved methods of medication and changes in

care and management. Beginning in 1936, the calf lots were cultivated and kept free from calves several months each year in an effort to control infection. During this time, calves were moved to fresh ground that had been free from cattle for several months. This program was followed for about 3 years. Beginning in 1939, calves that showed symptoms of pneumonia and scours were routinely treated with the sulfa drugs that proved most efficacious, and losses from these two causes were greatly reduced. The change to fresh ground was discontinued after sulfa therapy proved effective in reducing the death losses from certain infectious diseases. It is concluded that improved therapy and changes to fresh ground explain the difference in mortality before and after 1936.

Causes of Death

An understanding of the specific causes of death should be helpful in analyzing mortality rates. Unfortunately, routine autopsies of all calves that died or were destroyed were not attempted until this study had been under way for some time. On account of holidays, Sundays, and occasional absence of the veterinarian, an autopsy upon each calf could not well be obtained. Most of the autopsies were performed by a member of the Division of Veterinary Science of the University of California. Even though only one veterinarian worked with the herd at any one time, over the period of years involved, many different veterinarians performed the autopsies. Table 5 shows the causes of death. If a calf was not treated by a veterinarian, and was not autopsied, the cause of death is classed as unknown. Had all the calves been autopsied, there would be less need for such a classification. Pneumonia took a heavy toll in all the inbreeding classifications. "Digestive Trouble" includes all types of diarrhea, enteritis, and bloat, but not the diagnosed cases of coccidiosis. Digestive disturbances accounted for more deaths than any one cause; coccidiosis for a small percentage of losses. The classification "non-specific infection" refers to all cases of acute infection not otherwise classified. It includes septicemia, infected parts such as a kidney or joint, and a few possible cases of calf diphtheria and encephalitis that were never confirmed by laboratory tests. The miscellaneous group contains two calves. The male in class III could urinate only with difficulty and died at 16 days. The female in class I was born prematurely.

TABLE 5
POST-NATAL DEATHS CLASSIFIED
CLASS OF INBREEDING

	I		II		III		IV	
	Number died	Mean days lived	Number died	Mean days lived	Number died	Mean days lived	Number died	Mean days lived
				JERSEY MALES				
Unknown	6	21.0	1	1.0	10	19.2	5	28.3
Pneumonia	5	40.8	2	61.0	4	56.0	2	42.5
Digestive trouble	—	——	1	18.0	4	35.0	1	31.0
Coccidiosis	—	——	—	——	2	40.0	1	11.0
Non-specific infection	—	——	1	34.0	—	——	2	63.5
Liver derangement	—	——	—	——	—	——	1	112.0
Miscellaneous	—	——	—	——	1	16.0	—	——
				JERSEY FEMALES				
Unknown	6	16.8	3	25.7	14	32.1	2	24.5
Pneumonia	3	23.0	6	53.7	6	33.3	2	31.0
Digestive trouble	7	18.4	11	42.3	3	37.3	3	71.5
Coccidiosis	1	34.0	—	——	—	——	3	47.0
Non-specific infection	1	40.0	1	10.0	—	——	3	68.0
Liver-derangement	—	——	—	——	3	91.6	2	82.5
Heart abnormality	—	——	1	4.0	—	——	1	1.0
Miscellaneous	1	2.0	—	——	—	——	—	——
				HOLSTEIN MALES				
Unknown	—	——	—	——	3	18.0	—	——
Pneumonia	—	——	—	——	2	35.5	—	——
				HOLSTEIN FEMALES				
Unknown	—	——	1	1.0	—	——	—	——
Pneumonia	1	60.0	—	——	2	73.5	1	30.0
Digestive trouble	—	——	—	——	5	29.0	1	27.0
Non-specific infection	1	41.0	1	13.0	—	——	—	——

The calves that succumbed to a liver derangement are of extreme interest. They lived longer than any other group. As was mentioned in an earlier section, two calves that lived beyond the period of 4 months were nevertheless included in the group that died. They had enlarged livers and clinically, at least, resembled the others that died before 4 months of age with deranged livers; hence they all appear to belong to the same clinical group. The descriptions of the livers from post-mortem examination are not identical, and unfortunately, no livers were sectioned or studied in detail. All were described as "enlarged." There is sufficient evidence to indicate a liver derangement, but not to establish the specific type of pathology. The two

deaths from congenital heart abnormalities occurred soon after birth, from 1 to 4 days of age.

Mortality That May Be Conditioned by Specific Lethal Genes

Specific lethal genes may account for certain types of the mortality in the higher classes of inbreeding. The 6 deaths which autopsies indicated to be caused by a liver derangement occurred in calves that were highly inbred. All are from inbreeding classes III and IV, which are from sire-daughter matings; and 5 were sired by 370A—a fact which strongly suggests that heredity may be responsible for these fatalities. Furthermore, the results of using 370A in sire-daughter matings indicates that a single recessive autosomal gene conditions the liver anomaly and that he is heterozygous. The sixth calf that died from the liver derangement was sired by 333C from a sire-daughter mating. Possibly this sire is also heterozygous for a recessive autosomal gene that conditions the liver disorder. Sires 370A and 333C are related, and both could have obtained the conditioning gene from a common ancestor.

It is unfortunate that more information was not obtained concerning the pathology of the liver disorder. The genetic ratios from the sire-daughter mating, however, make tenable the hypothesis of a single autosomal recessive conditioning gene—at least until more conclusive data can be obtained.

The two calves that died from a congenital heart anomaly were sired by 370A. One was from a sire-daughter mating, and the other had an inbreeding coefficient of 0.195 (see table 5, Jersey females inbreeding classes II and IV). One of these calves had a rather complete autopsy. The symptoms indicated an abnormal heart, and a stethoscopic examination confirmed that diagnosis. The calf was killed for autopsy at 4 days of age. It was found that the foramen ovale and interventricular septum failed to close; the result was anoxemia and weakness. The other calf died on the day of birth and was not autopsied; the veterinarian who examined it led the authors to assume that both calves suffered from the same ailment. The results of using sire 370A in sire-daughter matings does not give conclusive proof that the heart ailment is conditioned by heredity. There were 2 calves that died at 3 and 7 days of age from unknown causes and 2 others were stillborn. Death of all 4 could have been caused by the heart anomaly.

Approximately 60 calves were produced by sire-daughter matings but only one is definitely known to have manifested the heart ailment. If the heart anomaly has an hereditary basis, it is probable that a single autosomal recessive gene is responsible. All the information on the liver and heart abnormalities has been presented. Admittedly, there is insufficient pathological or genetic evidence to establish conclusively specific lethal genes as causative agents. If the sires now being used prove to transmit the two anomalies, it should be possible eventually to confirm or reject the tentative conclusion.

It is possible that other specific lethal genes may be causing mortality. Careful autopsies and other tests might have revealed lethals that interfere with physiological processes, without obvious morphological changes.

Mortality That Cannot be Accounted for by Specific Lethal Genes

There is no clear-cut explanation for the deaths in the higher-inbred groups that are attributed to penumonia, digestive trouble, coccidiosis, and non-specific infection. It is illogical to suspect that specific lethal genes are involved in the mortality from any of these symptoms: as was shown in an earlier section, among the progeny of one sire, within one inbreeding class and sex, viability can be increased by improved medication and management. This finding does not deny the action of specific genes in increasing mortality in the higher classes of inbreeding. It indicates that if genes are involved, they reduce vigor and make the calves less well fitted to survive. Just how genes may interact to cause lowered vitality should prove to be an intriguing physiological problem.

Post-natal Mortality of Progeny of Individual Sires

Most of the sires used produced progeny in only one inbreeding class. Six of them, however, had progeny in nearly all the classes of inbreeding. The information regarding the offspring of these sires is summarized in table 6. None of the sires produced enough progeny in individual classes of inbreeding to warrant giving the standard errors of the means, although some that were significant have been discussed in an earlier section. Table 6 shows the mortality among the progeny of the different sires. Since the numbers are small, most of

TABLE 6

POST-NATAL MORTALITY IN RELATION TO BIRTH WEIGHT WITHIN THE SAME SEX AND CLASS OF INBREEDING AMONG THE PROGENY OF INDIVIDUAL JERSEY SIRES

Bull	I Live births	I Mean birth weight	I Mortality	II Live births	II Mean birth weight	II Mortality	III Live births	III Mean birth weight	III Mortality	IV Live births	IV Mean birth weight	IV Mortality
					CLASS OF INBREEDING							
					MALES							
320A	15	63.0	0.27	5	60.5	0.00	8	59.3	0.37	—	——	——
300C	1	48.0	0.00	—	——	——	3	51.0	0.00	—	——	——
300D	22	59.5	0.09	1	53.0	0.00	4	55.0	0.00	—	——	——
333C	3	61.0	0.00	1	70.0	0.00	5	65.0	0.40	6	59.6	0.33
370A	21	67.2	0.19	6	67.5	0.00	17	64.5	0.29	10	63.0	0.50
386A	2	74.0*	0.00	2	57.0	0.50	6	56.5	0.67	—	——	——
433A	4	62.3	0.25	7	61.9	0.57	5	53.0	0.80	—	——	——
482A	—	——	——	3	50.5	0.00	1	50.0	1.00	—	——	——
					FEMALES							
300C	11	55.6	0.18	—	——	——	8	49.9	0.37	2	52.0	0.50
300D	33	48.0	0.12	1	54.0	0.00	14	45.2	0.21	3	41.5	0.33
320A	21	55.3	0.09	11	43.9	0.36	9	52.3	0.67	—	——	——
333C	4	62.0	0.00	1	——	0.00	7	58.7	0.00	16	60.2	0.31
370A	43	58.7	0.12	13	58.2	0.46	29	57.7	0.17	9	57.6	0.44
386A	5	56.7	0.00	6	54.7	0.17	5	51.2	0.40	—	——	——
433A	4	51.5	0.25	12	54.4	0.25	4	52.5	0.50	6	55.7	0.83
482A	—	——	——	6	45.8	0.50	6	53.3	0.17	2	40.0*	0.00

*Based on one calf

the differences are not statistically significant. Since birth weight, or the variation in birth weight, is apparently related to mortality in some manner, mean birth weights are given. As was mentioned earlier, occasional calves escaped weighing at birth, so that the mean weight given is not always based on the total number shown in the table. Within a given class of inbreeding, the birth weights of males are consistently higher than those of females, and the differences are statistically significant. Within the two highest classes of inbreeding, the mortality of males is greater than that of females but the difference is not significant in either case. When classes III and IV are combined the difference in mortality between the sexes is hardly significant. In the higher coefficients of inbreeding males may have a slightly greater mortality than females.

Since none of the sires produced enough progeny in any of the higher inbreeding classes for a satisfactory analysis of mortality, classes II, III, and IV were combined in the analysis of individual sires. Only six sires produced enough inbred progeny to warrant the X^2 analyses

shown in table 7. Sires 370A and 433A were the only ones that produced enough males for an analysis. The expected mortality is calculated from the mortality observed for class 1, males and females, which is 0.12 (table 3).

As table 7 shows, the inbred progeny of individual sires differ greatly in mortality. The inbred female progeny of 300D showed low mortality, and the deviation from the expected is not significant. Sire 482A is an inbred son of 300D from a sire-daughter mating; and here, again, the mortality is low and the deviation is not statistically significant. The deviation in mortality of the inbred progeny of 333C from the mean of the class I control group is not significant. From the inbred progeny of each of the sires 300D, 333C, and 482A, the mortality is higher than that of the control group. When all the progeny of the above 3 sires are combined, a X^2 test reveals that the deviation from the control group is statistically significant at the 1 per cent level. Evidently inbreeding increases the mortality in the progeny of these three sires, but numbers are insufficient to show a statistically significant deviation when each sire is considered individually.

TABLE 7

PROGENY MORTALITY OF INDIVIDUAL SIRES COMPARED INBREEDING CLASSES OF II, III, AND IV COMBINED AND CALCULATED ON THE EXPECTANCY OF THE MEAN MORTALITY OF CLASS I

Sire	Sex	Fate	Observed	Expected	X^2
300D	Females	Lived	14	15.750	1.55[1]
		Died	4	2.250	
320A	Females	Lived	10	17.500	25.71[2]
		Died	10	2.500	
333C	Females	Lived	13	15.750	3.84[1]
		Died	5	2.250	
370A	Females	Lived	35	44.625	16.61[2]
		Died	16	6.375	
370A	Males	Lived	23	29.073	11.66[2]
		Died	10	3.927	
433A	Females	Lived	12	19.250	21.84[2]
		Died	10	2.750	
433A	Males	Lived	5	10.572	23.90[2]
		Died	7	1.428	
482A	Females	Lived	10	12.250	3.306[1]
		Died	4	1.750	

[1]Not significant
[2]Significant at 1 per cent level

Sires 320A, 370A, and 433A produced enough inbred progeny for X^2 tests. Sires 370A and 433A produced enough male offspring for tests in addition to the females. In all cases the mortality of the inbred group is statistically greater than that of the controls. According to these studies, mortality increases with the degree of inbreeding, but the progeny of some sires show higher mortality than others. There was sufficient evidence to show, at least tentatively, that 370A might be heterozygous for two specific lethal genes, one affecting the liver, the other the heart. The mortality of inbred progeny of sires 320A, 370A, and 433A is rather high. All three sires are closely related. Bull 370A is a son of 320A, and 433A is also a son from a sire-daughter mating. If 370A is heterozygous for the two lethal genes suspected, possibly 320A and 433A carried the same genes. Unfortunately, routine autopsies were not being made while 320A and 433A were used as herd sires; and if deaths were caused by the liver or heart ailment in progeny of these sires, there was little chance of detecting such cause.

Discussion

The outstanding features of the study involve the relation of degree of inbreeding to mortality and the relation of certain environmental factors such as care, management, and medication to mortality. Without question, mortality increases with the degree of inbreeding, but sanitary conditions and improved therapy reduce the mortality.

Just how inbreeding increases mortality is not always clear. Specific lethals such as genes which cause liver and heart anomalies are apparently involved in some instances. Other evidence indicates that mortality sometimes results from a general reduction of vigor, which in turn, presumably may be caused by nonlethal recessive genes or unfavorable gene combinations. It is possible that a type of multiple allelic relationship somewhat similar to that suggested by East (1936) to account for the inability to fix heterosis into a true breeding state may be operating to cause the increased mortality in some cases. This seems to be the simplest explanation of the results where no lethals are involved and lack of vigor or low viability appears to be the cause of death. Some of the mortality may also be caused by an allelic series which operates in a similar manner to the T (Brachy) series in the mouse (Dunn, 1937). It may be possible to determine even-

tually whether or not a heterozygous relationship conditioning vigor accounts for certain types of mortality.

Lowell and Hill (1940) studied extensively the mortality rates of dairy calves in England and Wales. These workers did not take inbreeding into account. The percentage of abortions they reported was higher than that of our class I control group. The percentage of stillbirths was about the same. The percentage of mortality after birth was lower. The total mortality that they observed was 14.3 per cent. This is rather close to our observations for the combined Jerseys and Holsteins for the class I control group.

The relation of birth weight to inbreeding and mortality is a most interesting one. Some of our results, but not all, appear to parallel—at least, to a certain degree—those of Kolesnik (1939). Unfortunately, the authors have not yet obtained a translation of Kolesnik's important paper and have had to depend on the English summary. Perhaps the California and Russian data have more in common than the summary indicates. Both studies appear to have one finding in common—that mortality is correlated in some way with birth weight. The data of Kolesnik indicate that the lower the birth weight, the higher the mortality, whereas the California data indicate that it is the variance in birth weight, either high or low, that is positively correlated with high mortality. The studies of Kolesnik, Dickerson (1940), and the data presented here provide ample evidence that individual bulls sire progeny of a characteristic birth weight, heavy, medium, or light. In the California data, however, the bull that sired calves with the lowest mean birth weight manifested the lowest mortality; and certain other sires produced calves with high birth weight which manifested high mortality. The variance in birth weight is positively correlated with mortality. Extremes in birth weights are primarily attributed to segregation and homozygosity, and these conclusions are in accord with those of Kolesnik. King, in her extensive studies with the rat (1918a, 1918b, 1918c, 1919), found that inbreeding accompanied by rigid selection did not affect mortality. As the degree of inbreeding increased, there was an increase in litter size and longevity. Wright (1922a, 1922b), on the other hand, found that the degree of inbreeding affected vigor and mortality in the guinea pig. On the whole, inbreds were less viable and more readily succumbed under slightly adverse environmental conditions. For example, in the control stock which

was not inbred, litter sizes from three to six could be successfully raised, while in the inbred stocks a litter size of three or more was greatly handicapped and had little chance of surviving. In the inbred families, litter sizes of two or one had the best chance.

Even though all the calves studied were from single births, adverse environmental conditions seem to affect inbred calves and guinea pigs alike in increasing the mortality. To express the matter in other terms: improved environmental conditions, such as reduction in litter size of the guinea pig, more sanitary environment such as plowing the corrals or improved medication for calves, increased the survival capacity of the inbreds.

Wright (1922a), considering inbred families, cites examples of guinea pigs that are unusually large at birth but either are stillborn or die early. He attributes death perhaps to difficulties in parturition. There is no doubt that an unusually large fetus may cause dystocia. Since our data clearly show that inbred calves with extremes in birth weight, either high or low, succumb more readily than calves near the mean weight, it is possible that the same situation may occur in guinea pigs. The gestation periods of all the calves in our study were carefully checked, and only one live calf born prematurely was included. The problem of determining the effect of birth weight on mortality would be more difficult in guinea pigs, since size of litter and length of gestation period are important factors affecting size, but it can be done. It does seem important to determine whether or not genetic segregation is correlated with birth weight in relation to mortality among inbred progeny. If such a correlation generally exists, it may provide definite new methods to attack the problem of loss of vigor and increased mortality as a result of inbreeding.

There are many published reports concerning the results of inbreeding in swine. No attempt will be made here to review the literature in detail. It is interesting to compare the results obtained for cattle with those reported for swine. A few of the published reports indicate that inbreeding does not cause a decline in vigor or affect the mortality. Hughes (1933) worked with Berkshires and employed brother-sister matings for the most part. He obtained inbreeding coefficients (Wright's) of approximately 0.75. There was no decline in litter size nor increase in mortality after birth. Hughes informs the authors (personal communication) that there has since been a decline in vigor and

that possibly the line cannot be maintained. Godbey and Starkey (1937) describe an inbred line of Berkshires which has not suffered any serious decline in vigor or increased mortality as a result of inbreeding. Most other investigators that have practiced inbreeding in swine report a loss of vigor and increased mortality. The size of the litter farrowed may be reduced, or the litter size may remain the same, but there may be a substantial increase in mortality after birth so that the number of pigs weaned is greatly reduced. All reports indicate that increased mortality is the most serious problem to be surmounted in a swine-inbreeding program. These general conclusions are amply supported by studies of Chester Whites in the Bureau of Animal Industry of the U. S. D. A., by McPhee, Russel, and Zeller (1931) and Hetzer, Lambert, and Zeller (1940); in the Poland-China herd at the Minnesota Station by Hodgson (1935) and by Winters et al (1943). The higher degrees of inbreeding increased the mortality in an experiment with Poland-Chinas reported by Wilham and Craft (1939), who used half-brother X half-sister matings. In the first generation of inbreeding, 76 per cent of the pigs lived to weaning, but in the eighth generation only 44 per cent did so. Craft (1943), reviewing the research of the regional swine laboratory, concludes that inbreeding causes a decline in vigor and increases mortality in most strains of swine, though in some lines, the decline is slight. Evidently vigor and mortality among inbred calves parallel rather closely those observed in guinea pigs and swine.

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Summary

The University of California Dairy Cattle Breeding Experiment has been underway for about 25 years. This paper analyzes the factors that affect abortions, stillbirths, and mortality to 4 months of age. Only single births from normal parturitions were included. The 775 Jersey and 258 Holstein calves involved were kept under standard

conditions of feeding and management. Their coefficients of inbreeding were calculated and each was then placed in its proper inbreeding class. Class I, the control group, contained all animals whose coefficient of inbreeding was 0 to 0.1249, class II from 0.125 to 0.2449; class III from 0.245 to 0.3749; and class IV, 0.375 and over.

Although there was a slight excess of males in some classes, yet in general male and female births were approximately equal in each class, as would be expected if there is no differential mortality during the gestation period. The percentage of abortions in the control group was just as great as in the higher classes of inbreeding. It is concluded, therefore, that the degree of inbreeding has little or no effect on the number of abortions. The percentage of stillbirths increased with the degree of inbreeding, but, since the total numbers of stillbirths were relatively few, the increase is not statistically significant.

Both genetic and environmental factors were found to influence mortality. Total mortality, which includs abortions, stillbirths, and deaths after birth up to 4 months of age, increases with the degree of inbreeding in Jersey females. This is also true for Holstein female calves, but, because of fewer numbers and certain influences of the environment upon mortality, the effect is less marked.

The mortality to 4 months of age for calves born alive increases with an increase in the degree of inbreeding. The higher the degree of inbreeding, the higher the mortality. This applies to Jerseys of both sexes. Jersey males manifest a significantly greater mortality than females in inbreeding classes III and IV, but apparently because of limited numbers the difference is just below the level of significance. Under the experimental conditions, within the same sex and inbreeding class the Jersey calves were less viable than the Holstein. Individual bulls sire progeny of a characteristic birth weight—light, medium, or heavy—but there is no evidence of a correlation between characteristic birth weight and mortality.

Within the same breed, sex, and class of inbreeding, when the progeny of all the sires are combined, there is no significant difference in the mean birth weight between calves that lived and calves that died. Calves with extremes in birth weight, either light or heavy, succumb more readily than calves near the mean weight. Significant differences in variance of birth weight between surviving and non-surviving calves were observed in about half the classes that had adequate numbers.

A significant difference was also observed within the same sex and inbreeding class in the progeny of some sires.

The percentage of mortality varied among the inbred progeny of the different sires. Inbreeding apparently increased the mortality in the progeny of all sires, but the increase over the controls was not significant in the progeny of three of the sires. The mortality among the inbred progeny of all the other sires that had sufficient numbers of offspring was significantly greater than in the controls. It was tentatively concluded that two different lethal genes, conditioning anomalies of the liver and heart, but with no external morphological effects, may have been responsible for deaths in the inbred progeny of two of the sires. These two genes, however, are insufficient to account for all the mortality observed. It seems that more subtle genetic relationships or interactions may be involved in causing most of the mortality.

In inbred calves, the proportion of mortality that cannot be attributed to specific lethal genes was greatly influenced by management and medication. The mortality was reduced by keeping the calves on fresh clean ground that had been free from cattle for several months, and by the use of sulfa therapy in the treatment of certain infectious diseases.

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